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WO 01/45734 A1

(54) Title: CHEMICALLY-PROGRAMMABLE IMMUNITY

(57) Abstract: The present invention is related to methods and compositions that are capable of immediately immunizing a human or animal against any molecule or compound. The present invention comprises an immunity linker molecule with at least two sites; (1) a first binding site that binds to an immune system molecule in a human or animal that has been preimmunized against the first binding site, and (2) one or more second binding sites that bind specifically to a desired compound or molecule. The first binding site and the second binding site(s) are linked by a linker portion of the molecule.

CHEMICALLY-PROGRAMMABLE IMMUNITY

FIELD OF THE INVENTION

The present invention relates to methods and compositions for providing immediate immunity to any desired antigen. The present invention also provides methods and compositions for treating a wide variety of diseases without having to wait for an immune response to be mounted by the human or animal being exposed to the disease.

BACKGROUND OF THE INVENTION

The term "antigen" is defined as anything that can serve as a target for an immune response. The immune response can be either cellular or humoral. The term "vaccine" is defined herein as a suspension or solution of antigenic moieties, usually consisting of infectious agents, or some part of the infectious agents, that is injected into the body to produce active immunity. The antigenic moiety making up the vaccine can be either a microorganism or a natural product purified from a microorganism, a synthetic product or a genetically engineered protein, peptide, polysaccharide or similar product. The term "cell mediated immunity" is defined as an immune response mediated by cells rather than by antibody. It includes, but is not limited to, delayed type hypersensitivity and cytotoxic T cells. The term "adjuvant" as used herein is any substance whose admixture with an injected immunogen increases or otherwise modifies the immune response. A "hapten" is defined herein as a substance that reacts selectively with appropriate

antibodies or T cells, but the hapten itself is usually not immunogenic. Most haptens are small molecules or small parts of large molecules, but some macromolecules can also function as haptens. The term "conjugation" is defined herein as the covalent or other form of linking two or more molecules. It can be accomplished either by chemical means or *in vivo* by biologic means such as genetic engineering.

The process of immunization has been used for over a hundred years to protect humans and animals against disease. The process generally comprises injecting an antigen that is related to the pathogen in the human or animal and waiting an appropriate amount of time, allowing the human or animal in which the pathogen was injected to mount an immune response. The time required for mounting an immune response normally is between approximately two weeks and several months for most antigens. In most cases, a booster administration of the antigen is required to maintain the immune response. This booster is normally given weeks or months after the initial administration of the antigen. Thus, immunization is of little use for immediate treatment of a disease.

A separate immunization procedure must be made for each pathogen, although in some cases several antigens are included in a single vaccine. Every immunization carries with it a certain amount of risk that must be considered before any immunization is recommended on a wide-scale basis.

What is needed is a method of immunizing a human or animal that can result in an immediate immune response. In addition, a method of immunizing a human or animal by a single immunization would greatly reduce the inherent risks in the vaccination procedure.

SUMMARY OF THE INVENTION

The present intention provides methods and compositions for the immediate and specific immunization of a human or animal against a pathogen or other undesired substance. The present invention, in one embodiment, is designated an "immunity linker molecule" and comprises a molecule with multiple sites; a first binding site on the compound that is antigenic and is capable of mounting an immune response in a human or animal. After immunization of the human or animal, first binding site will then bind specifically to an antibody or other immune molecule that was induced by the immunization process. The molecule has a second binding site or sites that are capable of binding to one or more designated compounds. The present invention also includes a compound that contains only the first binding site or immunogenic site that is present in the immunity linker molecule. This compound that contains only the first binding site or antigenic site is designated herein as "the immunizing molecule".

According to the present invention, the immunity linker molecule can be made in several ways. The immunizing molecule with the first binding site can be physically linked or conjugated to the molecule with the second binding sites to the pathogen or other undesired substance. In another embodiment, the immunity linker molecule can be produced or manufactured as a single molecule containing the first binding site or immunizing site and the second binding sites. The immunity linker molecule can be any type of compound including protein, nucleic acid or a combination thereof. The first binding sight can be a hapten that is conjugated to a larger molecule.

In practicing the present invention, the human or animal is first immunized conventionally against the immunizing molecule. This process includes administering the molecule to the human or animal and then waiting an appropriate amount of time for an immune response to be mounted in the human or animal. If necessary, the immunizing molecule can be administered with an adjuvant and/or a booster may be given to the animal at appropriate

times. These methods of immunizing a human or animal are well known to one of ordinary skill in the art. The human or animal that has been immunized against the immunizing molecule now has antibodies that will bind the immunizing molecule when it is present in the blood or other fluid.

When the preimmunized human or animal is challenged with a pathogen or toxic substance, an immunity linker molecule that contains a binding site to the pathogen or toxic substance is administered to the human or animal. The immunity linker molecule binds at one site to the antibody that was previously induced, and binds to the pathogen at the second site thereby providing an immune complex of the antibody bound to the immunity linker molecule which is now bound to the pathogen. The body now recognizes the immune complexes and processes them in a normal manner.

Accordingly, it is an object of the present invention to provide a method and composition for the immediate and specific immunization of a human or animal.

It is yet another object of the present invention to provide a method and composition for immediately immunizing an immunologically naive human or animal.

It is another object of the present invention to provide a method and composition that enables one to quickly and easily select a desired antigen and immediately immunize the human or animal against that antigen.

Another object of the present invention is to provide a method and composition that will only require a single immunization to protect against a wide variety of pathogens and toxic substances, thereby reducing the risks of multiple vaccinations.

Yet another object of the present invention is to provide a method and composition that will allow health care professionals to immediately immunize a patient against a wide variety of pathogens and/or toxins.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 illustrates the structure of the immunity linker molecule.

Figure 2 illustrates the immunity linker molecule bound at one site to an antibody and, at a second site, to a desired molecule, thereby forming an immune complex .

DETAILED DESCRIPTION OF THE INVENTION

The present invention is related to methods and compositions that are capable of immediately immunizing a human or animal against any molecule or compound. The present invention comprises an immunity linker molecule with at least two sites; (1) a first binding site that binds to an immune system molecule in a human or animal that has been preimmunized against the first binding site, and (2) one or more second binding sites that bind specifically to a desired compound or molecule. The first binding site and the second binding site(s) are linked by a linker portion of the molecule.

The present invention comprises methods and compositions for the immediate and specific immunization of a human or animal against a pathogen or other undesired substance. According to the present invention, a human or animal can be immediately immunized against a chosen antigen simply by administering to the human or animal the immunity linker molecule with the appropriate second binding site. According to the present invention, one can provide immediate immunity to any chosen antigen on the basis of the pre-existing immunity to the immunizing molecule by administration of a synthetic chemical linker.

In practicing the present invention, the human or animal is first immunized conventionally against the immunizing molecule.

This process includes appropriately administering the molecule to the human or animal and then waiting an appropriate amount of time for an immune response to be mounted in the human or animal. The preferred method of administering the immunizing molecule is by injection. If necessary, the immunizing molecule can be administered with an adjuvant and/or a booster may be given to the animal at appropriate times. These methods of the immunizing a human or animal are well known to one of ordinary skill in the art. The human or animal that has been immunized against the immunizing molecule now has antibodies that will bind the immunizing molecule when it is present in the blood or other bodily fluid.

The immunizing molecule optionally can be administered with agents such as adjuvants, preservatives, diluents, emulsifiers, stabilizers, and other known components that are known and used in immunization procedures in the prior art. Any adjuvant system known in the art can be used in the composition of the present invention. Such adjuvants include, but are not limited to, Freund's incomplete adjuvant, Freund's complete adjuvant, polydispersed ß-(1,4) linked acetylated mannan ("Acemannan"), Titermax® (polyoxyethylene-polyoxypropylene copolymer adjuvants from CytRx Corporation), modified lipid adjuvants from Chiron Corporation, saponin derivative adjuvants from Cambridge Biotech, killed *Bordetella pertussis*, the lipopolysaccharide (LPS) of gram-negative bacteria, large polymeric anions such as dextran sulfate, and inorganic gels such as alum, aluminum hydroxide, or aluminum phosphate. A preferred adjuvant system is Freund's incomplete adjuvant. Another preferred adjuvant system is Freund's complete adjuvant. The method of immunization and the adjuvants used are not critical to the invention. Thus, any method known in the art can be used, and any adjuvant system known in the art can be used.

According to the present invention, immediate immunity to, for example, a pathogen, can be established in a human or animal that is immunologically naive to the pathogen by administering to

the human or animal that has been immunized against the immunizing molecule the immunity linker molecule that contains a binding site to the pathogen. The immunity linker molecule binds at one site to the antibody that was previously induced, and binds to the pathogen at the second site, thereby providing an immune complex of the antibody bound to the immunity linker molecule which is now bound to the pathogen. The body now recognizes the immune complexes and processes the complexes in a normal manner.

According to the present invention, the immunity linker molecule can be made in several ways. The immunizing molecule can be physically linked or conjugated to the molecule with the binding sites to the desired substance. In another embodiment, the immunity linker molecule can be produced or manufactured as a single molecule containing the multiple sites. In yet another embodiment, the immunity linker molecule consists of two active ends connected together by a rigid or flexible spacer such as a double helical region of RNA. The purpose of the spacer is to hold the two ends of the linker together, while preventing them from interacting.

The immunity linker molecule can be a protein, peptide, or nucleic acid molecule or any combination thereof, including, but not limited to, RNA molecules, DNA molecules or derivatives thereof. Preferably, the immunity linker molecule is comprised of RNA molecules and are produced according to the SELEX process. This process is described completely in the list of references attached hereto and are included herein by reference in their entirety.

The immunity linker molecule is shown schematically in Figure 1. The immunity linker molecule **10** comprises a first binding site **15** which is antigenic, a linking portion of the molecule **20** and a second binding site **35** that is capable of binding a specific molecule. The second binding site **35** site or sites are preferably aptamers that have been produced by the SELEX process. However, it is to be understood that the second binding site does

not have to be an aptamer, but can be any type of molecule that has the desired physical attributes, i.e., the second binding site being capable of binding to a specific molecule. It is to be understood that the immunity linker molecule can have more than one binding site to a single substance or can have multiple binding sites against multiple substances. The linking portion of the molecule links the first binding site **15** and the second binding site **35**. The linking portion **15** of the molecule can be double stranded nucleic acid, but other linking molecules can be used in the present invention. Figure 2 schematically shows the immunity linker molecule with an antibody **40** bound to the first binding site **15** of the molecule and a molecule **45** bound to the second binding site **35** on the immunity linker molecule **10**.

It is to be understood that the immunity linker molecule can be any type of molecule that is capable of being manipulated so that it is capable of (1) mounting an immunity response, and (2) binding a desired molecule or molecules. The preferred type of compound is nucleic acid or, preferably, modified nucleic acid such as 2'-fluoro- or 2'-amino-2'-deoxypyrimidine containing nucleic acids. Nucleic acids using these bases are much more stable than naturally occurring nucleic acids. (See Aptamers as tools in molecular biology and immunology, M. Famulok and G. Mayer, Cur.Top. Micro. Immunobiol., 1999, 243, 123-146.)

The immunity linker molecule can be administered to a patient intramuscularly, subcutaneously, orally, intravenously, or through the mucosal membranes. The immunity linker molecule can be use in immunizing a human or animal against a wide variety of substances, including, but not limited to, bacteria, fungi, viruses, toxic substances, and drugs.

The present invention is particularly useful in the military where troops may be unexpectedly exposed to a pathogen, toxin, or to a toxic chemical substance. Military personnel are preimmunized against the immunizing molecule, i.e., that portion of the immunity linker molecule that binds to the antibody. Then, if the military personnel are unexpectedly challenged with a

pathogen, the appropriate immunity linker molecule can be administered to the military personnel, thereby immediately protecting them against the pathogen or other toxic substance. The present invention can be used to prevent and/or treat organisms including, but not limited to, anthrax, dengue virus, or Marburg virus

Likewise, pharmacies can have a library of different immunity linker molecules available for a variety of different pathogens and toxic substances. If the patient has been preimmunized against the immunizing portion of the linker, then he or she will be immediately immunized against the pathogen or toxic substances.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in this disclosure.

Claims:

1. An immunity linker molecule, the immunity linker molecule containing at least one first site that binds to an immune system molecule and at lease one second site that binds to a desired compound or molecule.

2. The immunity linker molecule of claim 1 wherein the first site binds to an antibody.

3. The antibody of claim 2 wherein the antibody is from a human or animal that has been preimmunized against the first site.

4. The immunity linker molecule of Claim 1, wherein the desired compound or molecule is a microorganism.

5. The immunity linker molecule of Claim 4 wherein the microorganism is a bacteria, virus, or fungus.

6. The immunity linker molecule of Claim 1, wherein the compound or molecule is a drug.

7. The immunity linker molecule of Claim 1, wherein the immunity linker molecule is an aptomer.

8. A method of immunizing a human or animal against a molecule or compound comprising administering to the human or animal an immunity linker molecule, the immunity linker molecule containing at least one first site that binds to an immune system molecule and at lease one second site that binds to a desired compound or molecule.

9. The method of claim 8, wherein the human or animal has been preimmunized against the first site on the immunity linker molecule.

10. The method of Claim 8, wherein the immunity the desired compound or molecule is a microorganism.

11. The method of Claim 10 wherein the microorganism is a bacteria, virus, or fungus.

12. The method of Claim 8, wherein the compound or molecule is a drug.

13. The method of Claim 1, wherein the immunity linker molecule is an aptomer.

14. A method of preimmunizing a human or animal comprising immunizing a human or animal against a molecule that contains a first site that is immunogenic, the first site being present on an immune linker molecule, the immune linker molecule also containing at lease one second site that binds to a desired compound or molecule.

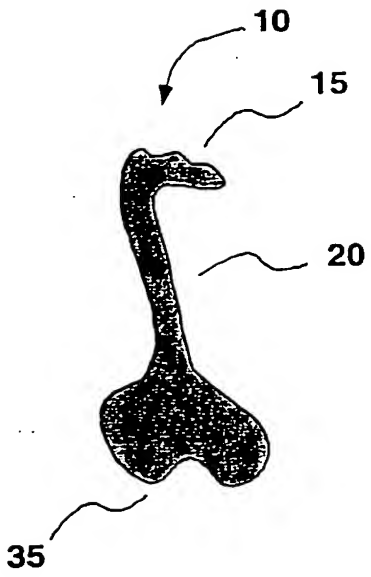

Figure 1

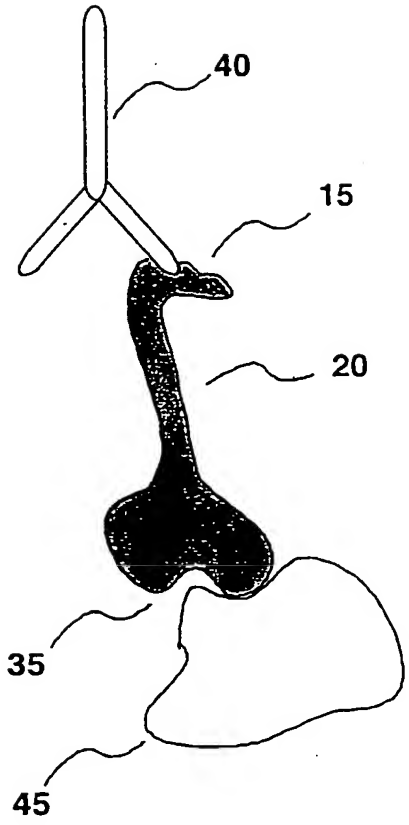

Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/35179

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 39/00, 39/385
US CL :424/184.1, 192.1. 193.1, 196.11, 197.11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 192.1. 193.1, 196.11, 197.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST: preimmun$.ti,ab,clm.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 5,204,449 A (PURI) 20 April 1993. See col. 1, line 53-col. 2, line 5; col. 2, lines 40-59; col. 3, lines 3-22 and 35-42; col. 5, lines 11-18.	1-6,8,10-12 ---------- 9
X ---- Y	US 5,017,558 A (VYAS) 21 May 1991. See col. 2, lines 16-27; col. 6, line 49-col. 7, line 5; claims 1 and 3.	14 ---- 9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 MAY 2001	Date of mailing of the international search report 05 JUN 2001
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Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/35179

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/35179

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-13, drawn to immunity linker molecules and methods of immunizing using these.

Group II, claim(s) 14, drawn to methods of preimmunizing.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the immunizing and preimmunizing methods of Groups I and II use different compositions, accomplish different purposes and can be conducted independently of one another. It is noted that the linker used in the first method has its first site as one that is directed to an immune system molecule, while the linker used in the second method has its first site as one that is immunogenic. Since the first sites of the two linkers thus differ in composition and in function, there is no special technical feature that provides for unity of invention.